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Influence of 2nd-degree AV blocks, ECG recording length, and recording time on heart rate variability analyses in horses

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Contents

Summary

Objectives: To assess the influence of 2nd-degree AV blocks (AVB) on RR interval-based heart rate variability (HRV) variables; to investigate the effect of using PP interval time series and of artifact filtering on HRV analyses; to investigate the influence of ECG recording length and time of recording; and to calculate day-to-day variability and reference intervals of HRV variables.

Animals: Thirty healthy adult horses.

Methods: RR and PP interval time series were extracted from 10-hour Holter ECGs and an automated filter was applied to the RR time series (RR_f). Time domain HRV variables were calculated based on RR, PP and RR_f time series and their relation to the number of AVBs was assessed. Hourly 10 and 60-min segments were extracted to investigate the influence of segment length and recording time on HRV variables.

Results: Variables of short-term HRV were significantly influenced by the number of AVBs when based on RR, but not when based on PP and RR_f time series. PP and RR_f-based HRV variables were in good agreement. The majority of HRV variables were influenced by recording time and ECG segment length. Day-to-day variability of HRV variables was low when based on 10-hour ECG recordings, but moderate to high when based on 60-min and 10-min recordings.

Conclusion: 2nd degree AVBs significantly influence conventional RR-based, but not PP- and RR_f-based time-domain HRV variables.

Key words: RR interval, PP interval, time domain, sinus arrhythmia, automated artifact filtering

Zusammenfassung

Studienziele: Untersuchung des Einflusses von AV-Blöcken 2. Grades (AVB) auf die RR-Intervall-basierte Berechnung der Herzfrequenzvariabilität (HRV), der Auswirkung einer PP-Intervall basierten Berechnung und Anwendung eines Artefakt-Filters auf HRV Analysen, des Einflusses von Aufnahmezeitpunkt und Aufnahmelänge und Berechnung der Tag-zu-Tag-Variabilität sowie Referenzwerten.

Tiere: 30 gesunde, erwachsene Pferde.

Methode: RR- und PP-Intervalle wurden aus 10-Stunden Holter EKGs berechnet und ein Artefakt-Filter wurde auf die RR-Intervalle angewendet (RR_f). Time Domain HRV Variablen wurden basierend auf RR-, PP- und RR_f-Intervall-Serien berechnet und deren Abhängigkeit von AVBs wurde beurteilt. Für jede Stunde wurden 10- und 60-Minuten Abschnitte zur Analyse des Einflusses von Aufnahmelänge und Aufnahmezeitpunkt berechnet.

Resultate: RR-Intervall-basierte Variablen der Kurzzeitvariabilität wurden, im Gegensatz zu PP- oder RR_f-Intervall-basierten Variablen, signifikant von AVBs beeinflusst. PP und RR_f-basierte Variablen stimmten gut überein. Die meisten HRV Variablen wurden von Aufnahmezeitpunkt und Aufnahmedauer beeinflusst. Die Tag-zu-Tag-Variabilität bei 10-Stunden Analysen war gering, hingegen mittel- bis hoch bei 60- und 10-Minuten Analysen.

Schlussfolgerung: AV Blöcke zweiten Grades haben einen signifikanten Einfluss auf RR-basierte HRV Berechnungen, nicht jedoch auf PP- und RR_f-basierte HRV Analysen.

Schlüsselwörter: RR, PP, Time Domain, Sinusarrhythmie, Artefaktkorrektur

Introduction[1]

Heart rate variability (HRV) analysis has been used in horses to assess vago-sympathetic balance in a wide variety of conditions and with animals exposed to various stimuli[1–32]. Because of a lack of widely accepted, standardized procedures, the studies involving HRV analyses in horses use different strategies of heart rate recording, data processing, HRV calculation and data interpretation, prohibiting direct comparison of HRV results. Only a few of the published studies declare in detail the methods used for HRV analyses. Generally, published studies are either based on surface electrocardiograms (ECGs) or heart rate monitor recordings (e.g. Polar). It is generally well accepted that abnormal beats (e.g. supraventricular and ventricular premature beats) and artifacts should be excluded from the RR interval time series to obtain a normal-to-normal (NN) interval time series representing sinus node activity, based on which the HRV analyses should be performed. However, depending on the available raw data, recordings can (for ECG recordings) or cannot (for RR interval time series obtained with heart rate monitors) be manually corrected for abnormal beats and artifacts. Automated filtering by some type of software algorithm is discussed as a valuable tool to speed up ECG analysis for artifact removal[33,34]. Software products capable of automated filtering are available[35,36], but the methods used by the automated filters are often not well described and the validity of HRV analyses based on filtered data is often unclear.

In horses, the occurrence of physiologic 2nd degree AV blocks (AVBs) is an influential factor affecting the results of HRV analyses. AVBs intermittently prolong the RR intervals and therefore result in increased HRV[37]. The frequency of 2nd-degree AVBs varies between horses and in the same horse over time[38]. Often times, similar to segments with pathologic arrhythmias, ECG segments containing 2nd-degree AVBs are excluded from the data processing to obtain a NN interval time series representing sinus node activity. However, this introduces some bias to the HRV analysis because these ECG segments might in fact represent phases of increased vagal tone and contain important information about vago-sympathetic balance. It is currently unknown to what extent physiologic 2nd-degree AVBs influence HRV analyses in horses.

Therefore, the objectives of this study were to assess the influence of the number of 2nd-degree AV blocks on different time-domain variables of short-term, long-term, and overall HRV and to investigate whether HRV analyses based on PP interval time series (instead of RR interval time series) are feasible and able to reduce the influence of 2nd-degree AV blocks on HRV variables. Furthermore, an artifact correction filter included in a commonly used HRV analysis software package was investigated and filtered RR-based (RRf) were compared to unfiltered RR-based and to PP-based HRV variables. Using the PP interval time series, differences in HRV variables resulting from various ECG recording lengths and time of ECG recording overnight were evaluated and the day-to-day variability of HRV variables was assessed. Finally, reference intervals for PP-based and RRf-based HRV variables were calculated.

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Materials and methods

Study population

Healthy horses were recruited from horse stables in the surrounding area of the Vetsuisse Faculty, University of Zurich. All horses were privately owned and considered healthy based on history, physical examination, and ECG analyses. Horses with a recent history of fever, exercise intolerance, known heart disease, a heart murmur grade 3-6/6 on auscultation, or pathologic arrhythmia based on a 12-h Holter ECG were not included. The study was approved by the district Veterinary Office of the Canton of Zurich, Switzerland (Ref # 22/2014, Date of approval February 26, 2014). Owners' consent was obtained.

ECG Recording

Holter ECG recordings were conducted by a single operator (BE) using a recording device[2] offering a 12-bit resolution and a sampling rate of 500 Hz. It was mounted on the horses' back, padded with cotton wool and covered by an elastic, adhesive bandage. The ECG electrodes[3] were placed on the thorax in standardized locations to obtain a modified base-apex lead[39] (Figure I). The hair was not clipped before electrode placement. After instrumentation, successful operation of the device and quality of recordings was verified by visual inspection of the ECG using the telemetry mode of the ECG device. The quality of the ECG was considered to be sufficient if the baseline was stable; P, QRS, and T waves were clearly discernible; and the ECG tracing was not disturbed by excessive motion artifacts (i.e. when the horse was moving in the stall). The recordings were conducted in a standardized way starting before 8.00 pm and proceeding until 6.00 am or later the following day, resulting in recordings of at least 10-h duration. All recordings were taken in the horses' own box stalls and the owners were advised to leave the horses undisturbed until recordings were finished. No other restrictions were imposed and barn work continued as usual.

In a subset of five horses, ECG recordings were repeated twice on two different days for assessment of day-to-day variability of HRV analyses. All ECG data were recorded in digital raw data format and stored on an SD card.

Data processing

Data processing including all analyses and corrections were made by a single operator (BE). The digital ECG files were then imported into standard ECG analysis software (Televet[4]) and shortened to a standard recording length of 10 hours, starting at 8.00 pm and ending at 6.00 am. This time sequence was saved as ECG data file and subsequently processed in three different ways:

(1) RR interval time series were derived using the Televet[4] software. The quality of the recordings was first verified using the overview screen. RR interval analyses were then performed using the integrated equine RR analysis algorithm (Figure II). All RR intervals

[2] Televet 100, Engel Engineering Services GmbH, Heusenstamm, Germany

[3] Kruuse ECG electrodes, Jørgen Kruuse A/S, Langeskov, Denmark

[4] Televet 5.1, Engel Engineering Services GmbH, Heusenstamm, Germany

with a deviation of more than 20% compared to the preceding interval were marked and checked visually by the operator. Corrections of RR detection were made manually where necessary. All 2nd-degree AVBs were visually identified and counted. This way, a 100% detection rate of RR intervals and AVBs was ensured. The corrected RR time series (RR) were then exported into text files.

(2) PP interval analyses were performed using dedicated software including a customizable shape recognition algorithm (ecgAUTO[5]). The ECG data files were imported (Figure III) using a custom-made Televet-to-ecgAUTO data import module. The software's wave form identification algorithm was then used to identify P waves in the ECG recordings. This algorithm is primarily intended to identify R waves, but for this study P wave detection was enabled by building a waveform template where the P waves were marked as R peaks (Figure IV). The template was then used to identify of P waves during the automated analysis of the ECG data file (Figure V and VI). Manual correction of the analyses was not possible in the software. However, the analysis results were checked visually and the P wave detection rate was assessed by the operator. If necessary, additional waveform templates were created for differently shaped P waves and added to a waveform library (Figure VII). Multiple sequential analyses were performed using an iterative approach, each time adding another waveform template to detect previously missed P waves. This way, for each ECG data file (i.e. for each recording), an individual waveform library was created to optimize detection rate.

The time in minutes required for RR interval analysis in Televet and PP interval analysis in ecgAUTO was recorded. The number of PP intervals identified using the individual waveform library was compared with the total number of cardiac cycles (i.e. PP intervals) included in each analysis, which was calculated as sum of the number of RR intervals plus the number of AV blocks identified using the Televet software. The percentage of PP intervals correctly identified was then calculated. The PP time series (PP) was exported in a text file.

HRV analysis

Ten-hour time series text files (RR, PP) were processed using dedicated HRV analysis software (Kubios HRV[6]) for calculation of time-domain variables. A third set of variables was calculated by applying an artifact correction filter to the RR time series (RRf). This filter, set at the 'very-low' level (Figure VIII), uses a piecewise cubic spline interpolation method to eliminate all intervals 0.45 seconds longer or shorter than the local average interval length based on a heart rate of 60 beats/min[40]. An example of the effects of PP analysis and filtering of the RR time series on the variability and temporal distribution of NN intervals is shown in Figures IX.

The following HRV variables were calculated: Mean heart rate, mean of all normal-to-normal intervals (MeanNN, where NN corresponded to the corrected RR, PP, and RRf intervals), standard deviation of all NN intervals (SDNN), triangular index (TI, calculated as the integral of the NN interval histogram divided by the height of the histogram), standard

[5] ecgAUTO 3.3.0.2, emka TECHNOLOGIES, Paris, France

[6] Kubios HRV v2.1, University of Eastern Finland, Kuopio, Finland

deviation of the average of NN intervals in all non-overlapping 5 min segments (SDANN), square root of mean squared differences between successive NN intervals (RMSSD), and standard deviation quantifying the dispersion of data points in a Poincaré plot (Figure XI) perpendicular to the line of identity (SD1) and along the line of identity (SD2). Results were exported as text files for further data handling. For all repeated recordings, data processing and HRV analyses were performed in an automated fashion to avoid operator bias.

The SDNN and TI are considered variables representing overall HRV, SDANN, and SD2 are considered variables of long-term HRV, and RMSSD and SD1 are considered variables of short-term (i.e. instantaneous, beat-to-beat) HRV[41–43].

The PP interval analysis in the ecgAUTO software resulted in an artifact due to a technical phenomenon called 'oscillation'. This is an inherent limitation of the software algorithm and occurs because P wave recognition is based on templates defining the location of the markers for interbeat interval measurement. Because these templates have a specified length, certain interval lengths may artificially accumulate (Figure IX B). This causes a false peak in the PP histogram, which affects the calculation of triangular index and leads to a falsely low value. Therefore, for PP-based HRV analysis, the triangular index was recalculated manually after exclusion of the artificial peak. Because mathematically this phenomenon does not affect the other time-domain variables to a considerable extent, no other manual corrections were done.

For the PP time series, all HRV variables were not only calculated for the entire 10-h recording but also for every hour separately and for the first 10 minutes of every hour separately, to assess the influence of recording length and time of ECG recording on HRV variables and to evaluate day-to-day variability of variables.

Statistics

Statistical analyses were carried out using SigmaStat v3.5[7] and GraphPad Prism v6.07[8]. Unless stated otherwise, the level of significance was p=0.05.

The time required for RR and PP interval analyses, respectively, was compared using a Wilcoxon signed-rank test.

Linear regression analyses were performed to assess the relationship between the number of 2nd-degree AVBs occurring in the 10-h recording period and the corresponding HRV variables, calculated based on the 10-h RR, PP, and RRf time series. Regression slopes were compared pairwise between RR-based, PP-based, and RR_f-based variables, with the level of significance after Bonferroni correction for three comparisons being p=0.05/3=0.017.

Bland-Altman analyses were performed to calculate mean bias and 95% limits of agreement for comparison between PP-based and RRf-based HRV variables calculated on the 10-h time series[44,45]. Since two outliers were detected (see Figures XII, XIII and XIV), Bland-Altman analyses were repeated without these outliers. When the difference between methods was independent of the average value, the difference bias (PP-RRf) was used.

[7] SigmaStat v3.5, Systat Software GmbH, Erkrath, Germany

[8] GraphPad Prism v6.07, Graph Pad Software Inc, La Jolla, CA, United States

However, when there was a trend of a larger bias at higher average values (i.e. for RMSSD and SD1), the ratio bias (PP/RRf) was used.

The PP-based HRV variables were evaluated by two-way repeated measures ANOVA for changes over time and differences between segment lengths (10 min vs 60 min). Validity of the normality assumption was confirmed by assessment of normal probability plots of the residuals. No post-hoc multiple comparison tests were performed. Results were plotted together with the HRV variables calculated using the 10-h PP time series for comparison.

Day-to-day variability of PP-based HRV variables was quantified based on the repeated ECG recordings and HRV analyses of five horses. Variability was assessed for the entire 10-h time series and for selected 60-min and 10-min segments. One 60-min and one 10-min segment was randomly selected from the first 10-h recording of each horse and the corresponding segments of the subsequent recordings were then included in the analysis for each horse. One-way ANOVA with horses as the groups was used to calculate the within-subject variance for repeated measurements (residual mean square)[46]. The within-subject standard deviation (sw) was calculated as the square root of the residual mean square. Day-to-day variability was reported in two ways: (1) The within-subject coefficient of variation (CV) expressed as a percent value was calculated as CV = sw / mean × 100, to compare the variability among the various variables used in this study. The magnitude of variability was defined as follows: CV<5%, very low variability; 5-15%, low variability; 16-25%, moderate variability; >25%, high variability. (2) In addition, the absolute value below which the difference between two measurements will lie with 95% probability was estimated following the British Standards Institution (BSI) recommendations: BSI = $1.96 \times \sqrt{2} \times sw = 2.77 \times sw$ [46].

Reference intervals

The reference intervals for the time-domain HRV variables derived from 10-h PP and RRf time series were calculated using a dedicated software package (Reference Value Advisor[9]). Distribution of the data was checked using raw data box-and-whisker plots, histograms, and normal probability plots. Mean and SD of all variables were reported. The reference intervals were calculated using robust methods based on Box-Cox transformed data. The 90% confidence intervals (CI) of the limits of the reference intervals were determined using a bootstrap method.

[9] Reference Value Advisor 2.1, École Nationale Vétérinaire de Toulouse, Toulouse, France

Results

Population and recordings

Thirty healthy horses (18 geldings, 12 mares; 20 Warmbloods, one Freiberger, one Quarter Horse, two Trotters, six Icelandic horses) aged 14 ± 5.5 (mean ± SD) years and with a body weight of 572 ± 99 kg were recruited and met the inclusion criteria. Out of 30 recorded ECGs, eight contained no 2nd-degree AVB and 22 contained between 1 and 325 2nd-degree AVB over the 10-h recording period. The number of RR and PP intervals and the number of AVBs detected on the ECG recordings are summarized in Table 1. The estimated P wave detection rate ranged from 97% to 100% (median 99%). The time required for RR and PP interval analyses was 13 [5-43] min and 28 [5-92] min, respectively (median [range], $p<0.001$).

The group of horses with repeated ECG recordings included four Warmbloods and one Icelandic horse (three geldings, two mares; aged 17 ± 7 years; body weight 614 ± 159 kg). The time span between the first and the subsequent recordings was 4.4 ± 0.8 months, with the second and third recording obtained during two successive nights. The number of RR and PP intervals, the number of AVBs and the estimated P wave detection rate for repeated analyses are also listed in Table 1.

Influence of 2nd-degree AVBs

The influence of the number of 2nd-degree AVBs on RR-based, PP-based, and RRf-based HRV variables is illustrated in Figure 1. A statistically significant, moderate to strong positive association was found between the number of 2nd-degree AVBs and RMSSD and SD1 when calculated based on the RR time series (Figure 1 G and H). This effect was abolished when HRV variables were calculated based on PP and RRf time series. In fact, none of the HRV variables was associated with the number of 2nd-degree AVBs when calculated based on PP and RRf time series. The slopes of the regression lines of the PP-based and the RRf-based HRV variables were not significantly different for any of the variables.

Bland-Altman analyses, excluding two outliers, are summarized in Figure 2 and Table 3 and indicate good agreement between PP-based and RRf- based HRV variables. The two outliers originated from poor P wave detection and presence of marked sinus arrhythmia with sinus pauses (sino-atrial blocks), respectively (see Table 1 and Figures XII, XIII and XIV).

Influence of segment length and recording time

The influence of segment length and recording time on PP-based HRV variables is shown in Figure 3. Mean heart rate decreased and mean NN interval increased overnight. Significant differences between recording times were seen in SDNN, SDANN, SD2, RMSSD, and SD1, for both 10-min and 60-min segments, with increasing values overnight. When comparing HRV variables calculated using 10-min and 60-min ECG segments, significant differences were found in SDNN, TI, SDANN, SD2, with higher values during longer recordings.

Day-to-day variability

The data related to the day-to-day variability of PP-based HRV variables are summarized in Table 2. The results show that variability of repeated measurements was very

low (CV < 5%) for mean HR and mean NN and low (CV 5-15%) for all HRV variables when calculated based on 10-h recordings. Mean HR and mean NN showed low variability when based on 60-min and 10-min ECG segments. However, HRV variables based on 60-min and 10-min ECG segments showed moderate to high day-to-day variability.

Reference intervals

The reference intervals for the time-domain HRV variables derived from 10-h PP and RRf time series are summarized in Table 3. Two horses were eliminated from the analyses, as they were marked as outliers by the analysis software. These two horses represented the same horses that had been eliminated for Bland-Altman analyses (Figures XII to XIV).

Discussion

This study is the first to quantify the effect of physiologic 2nd-degree AVBs on conventional RR-based time-domain HRV variables in horses. It is also the first to show that automated PP interval analysis in routine surface ECGs from horses is feasible and, similar to artifact filtering, can eliminate the effect of 2nd-degree AVB and provide the basis for HRV analyses. Furthermore, it provides data on the effect of length and time of ECG recording and on day-to-day variability of PP-based HRV variables, indicating that repeatability of HRV variables in horses is markedly dependent on ECG segment length.

Influence of 2nd-degree AVBs

As the number of 2nd-degree AVBs increase, there is a marked effect on instantaneous, beat-to-beat HRV (expressed by RMSSD and SD1) when using conventional RR interval time series as a basis for HRV analyses (Figure 1). This effect can be abolished when HRV analyses are based on PP intervals series, which allow for assessment of primarily the sinus nodal function and appear to remove the influence of 2nd-degree AVBs. In addition to 2nd-degree AVBs, the occurrence of variable PQ intervals and 1st-degree AVBs, both of which may be seen in healthy horses, also needs to be considered. While affecting the RR time series, neither varying PQ intervals nor 1st-degree AVBs will influence PP time series. Elimination of this additional influential factor might in fact be a further advantage of using PP time series for HRV analyses. It is possible that some horses in the study population also showed both varying PQ intervals and intermittent 1st-degree AVB, which might have contributed to the differences seen between RR-based and PP-based HRV variables, but this was not further investigated in this study.

The results of this study indicate that automated PP interval analyses are feasible, but technically challenging and time consuming. They require sophisticated and expensive software that is not designed for routine clinical use. Detection of peak P might be affected by P wave conformation, which can vary over time[47–49] and which can be affected by heart rate and recording artifacts (Figure XIII). Also, PP interval analysis is not able to eliminate effects of sinus pauses or SA blocks on HRV analyses (Figure XIV). Therefore, filtering of conventional RR time series to remove the effect of 2nd-degree AVBs might be more applicable for many applications in clinics and research. In this study, PP-based and RRf-based HRV variables are in good agreement. Although filtering seems applicable for the use in a clinical setting, one has to keep in mind that filtering may not only eliminate 2nd-degree AVBs but will also remove marked sinus arrhythmia as well as sinus pauses and sino-atrial blocks (Figure XIV). Therefore, despite the fact that no significant differences between PP and RRf-based HRV analyses are seen in this study including a population of 30 horses, the effect of filtering must be critically assessed if an analysis is conducted in an individual horse. In general, filtering as described in this study might be applicable in the majority of horses, with the exception of those showing marked sinus arrhythmia.

Influence of segment length and recording time

In this study, ECG recordings were performed overnight in the horses own box stalls to reduce the influence of environmental factors on HRV analyses. The influence of ECG segment length and recording time was assessed using the PP time series.

It is known that HRV indices to a varying degree depend on recording length[50] and that only recordings of the same length should be compared[42]. In horses, the optimal

recording length used for time-domain HRV analyses is not known and probably depends on the question to be answered. Previous studies performed in horses often followed published recommendations[42] and used 5-min segments for time-domain analysis based on segment lengths used in humans[51]. However, because of the lower resting heart rate of the horse compared to people, 10-min segment lengths might be advantageous when HRV analyses are performed at rest[52]. In this study, segments with a length of 10 min, 60 min, and 10 h were analyzed and compared.

The results clearly show that all time-domain HRV variables are influenced by ECG recording length (Figure 3). Generally, HRV is lower the shorter the analyzed time segments are. This effect is most prominent for variables reflecting overall (SDNN, TI) and long-term HRV (SDANN, SD2). The SDANN cannot be calculated consistently based on 10-min recordings because of the low number of interbeat intervals. Therefore, recordings longer than 10 min are preferable for HRV analyses in horses.

Comparisons of HRV variables over time, throughout the course of the night, indicate that the time of recording is crucial for some of the variables. Mean HR decreases and mean NN increases over night, presumably associated with an increase in parasympathetic tone. Concurrently, an increase in overall HRV (expressed by SDNN), long-term HRV (expressed by SDANN and SD2), and short-term (instantaneous, beat-to-beat) HRV (including RMSSD and SD1) is seen. The TI is not significantly influenced by the time of recording. This is likely related to the geometric algorithm used for calculation of the TI[43] and suggests that the TI is more robust but may be less sensitive to detect changes in overall HRV over time. Conversely, the TI is influenced by segment length (see above) because the TI algorithm is basically a summation of intervals.

Day-to-day variability

In this study, we investigated the day-to-day variability of PP-based HRV analyses over a longer period of time. The results indicate that HRV variables based on 60-min and 10-min ECG segments are moderate to highly variable and are therefore not suitable to detect minor changes in HRV variables over time. The HRV variables based on 10-h recordings are less variable and may therefore be preferable for clinical applications. No conclusions can be made for recordings of different durations other than those used in this study.

Limitations

The study was based on a population of horses of different age, sex, and breed. Although these factors might play a role, it was beyond the scope of the study to investigate their influence on HRV variables.

Due to the large number of cardiac cycles that are to be included in HRV analyses, the measurements of NN intervals (i.e. RR and PP intervals) are largely based on automated peak detection algorithms that can only be partially verified by the operator. As a technical limitation, the software algorithms are only able to identify distinct waves on recordings with a favorable signal-to-noise ratio. The difference in signal amplitude predicts that P wave detection is technically more challenging than R wave detection. Hence, only clearly identifiable 2nd-degree AVBs with existence of a distinct P wave without QRS complex are able to be detected in the analyzed ECGs. However, consistent recording of distinct P wave morphologies can be difficult when using thoracic leads, because motion artifacts may affect recording quality and P wave morphology is variable and can change over time. The use of different lead positions, possibly even individualized to the animal, might allow improving the signal-to-noise ratio and increasing the P wave detection rate, but at the cost of losing standardization and possibly impairing automated RR detection and rhythm diagnosis. No alternate lead positions were used in this study. The lack of clearly identifiable P waves resulted in median estimated P wave detection rates of 99%, indicating that the automated P wave detection algorithm has its clear limitations. Another limitation inherent to the software algorithm is the phenomenon called ‘oscillation’ that has been discussed above.

Some limitations also need to be discussed in relation to the artifact filter that has been used in this study. Automated filtering is a straightforward software-based mechanism to eliminate aberrant RR intervals depending on a given algorithm. However, since the filter is applied to the imported RR time series and not to the original ECG recording, the operator cannot verify with certainty that the eliminated RR intervals are identified correctly and that only 2nd-degree AVB but not, for example, episodes with marked sinus arrhythmia are removed from HRV analysis. Therefore, the filer possibly introduces some bias by excessively reducing HRV. This could not be further investigated in this study. Another potential limitation concerning the automated filtering algorithm might be the fact that the filter algorithm is based on an average human heart rate of 60 beats/min, whereas horses usually have lower average resting heart rate. However, the analysis software does not allow modifying the filter. The lowest possible correction mode was chosen to avoid excessive filtering.

Conclusions

The results of this study show that 2nd-degree AVBs significantly influence conventional RR-based time-domain HRV variables in horses. Automated PP interval analysis in routine surface ECGs from horses is technically challenging but feasible, and it appears to be a suitable way to reduce the influence of 2nd-degree AVBs on time-domain HRV analyses. Alternatively, automated filtering of RR interval time series might provide a clinically applicable method to correct for occurrence of 2nd-degree AVBs. However, neither of the methods are without limitations and quality assurance is difficult.

Recording length and time of recording can significantly influence time-domain HRV variables and therefore need to be considered and standardized when recording ECGs for use in HRV studies. Day-to-day repeatability is improved when using longer ECG segments for PP-based HRV analyses.

This study provides some insight into a commonly encountered issue when applying HRV analyses to horses. However, HRV analyses are influenced by many different factors that were not all considered in this study: individual horse factors, environmental stimuli, equipment and ECG recording methods, methods of ECG interpretation, and type of software used for automated or manual data processing, filtering and HRV calculations. Additional research and a consensus regarding standardization of HRV analyses for horses are therefore required to ensure data quality and compare results between different studies.

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Tables

Table 1. Number of RR intervals, PP intervals and 2nd-degree AV blocks in the 10-h overnight recordings of 30 horses, including the repeated recordings of five horses.

Horse	Number of RR intervals (#RR)	Number of PP intervals (#PP)	Number of 2nd degree AVBs (#AVB)	Estimated P wave detection rate[a]
1	21877	21714	1	0.99
2	21914	22060	185	1.00
3	23946	23821	44	0.99
4	26781	26487	0	0.99
5	22754	22603	81	0.99
6	24210	23797	25	0.98
7	22237	22302	149	1.00
8	18556	18330	0	0.99
9	28869	28431	0	0.98
10[b]	27623	26861	4	0.97
11	27368	27089	0	0.99
12	26286	26066	0	0.99
13	20916	20707	35	0.99
14	23574	23366	0	0.99
15	17644	17542	1	0.99
16	20382	20103	9	0.99
17	22698	22427	1	0.99
18	21173	20918	7	0.99
19[b]	19967	19787	6	0.99
20	20646	20415	41	0.99
21	21936	21757	5	0.99
22	20895	21070	308	0.99
23	23097	22665	325	0.97
24	21096	20958	0	0.99
25	20882	20613	67	0.98
26	20101	20019	4	1.00
27	20239	20107	16	0.99
28	25619	25419	3	0.99
29	26290	25968	0	0.99
30	21685	21442	9	0.99
Mean ± SD	22708 ± 2817	22494 ± 2730		
Median (range)			5.5 (0 – 325)	0.99 (0.97 – 1.00)
2nd recording				
15	16713	16360	68	0.97
22	21376	20874	125	0.97
28	22789	22394	11	0.98
29	23979	23496	16	0.98
30	23054	22634	0	0.98
3rd recording				
15	16614	16636	9	1
22	20568	20191	363	0.97
28	24269	24062	7	0.99
29	25557	25097	1	0.98
30	22309	21995	0	0.99

[a] Estimated P wave detection rate = #PP / (#RR + #AVB)

[b] Horses that acted as outliers and were removed from Bland-Altman analyses and reference interval calculation.

Table 2. Influence of ECG segment length on day-to-day variability of PP-based HRV variables.

Variables	Units	10-min ECG segment				60-min ECG segment				10-h ECG segment			
		Missing Data	Mean±SD (n=15)	CV	BSI	Missing Data	Mean±SD (n=15)	CV	BSI	Missing Data	Mean±SD (n=15)	CV	BSI
MeanNN	ms	0	1757 ± 184	11	509	0	1720 ± 113	7	314	0	1667 ± 61	4	170
MeanHR	1/min	0	36 ± 5	13	12	0	37 ± 3	8	9	0	38 ± 2	5	5
SDNN	ms	0	180 ± 46	25	126	0	218 ± 38	17	106	0	247 ± 15	6	42
TI	-	0	23 ± 4	17	11	0	33 ± 6	18	17	0	60 ± 9	15	25
SDANN	ms	10[a]				0	121 ± 39	32	107	0	175 ± 21	12	58
SD2	ms	0	240 ± 61	25	168	0	294 ± 58	20	160	0	339 ± 23	7	62
RMSSD	ms	0	111 ± 56	50	154	0	127 ± 36	29	100	0	118 ± 19	16	52
SD1	ms	0	78 ± 39	50	109	0	90 ± 26	29	71	0	83 ± 13	16	37

Abbreviations: SD1, standard deviation quantifying the dispersion of data points in a Poincare´ plot perpendicular to the line of identity; SD2, standard deviation quantifying the dispersion of data points in a Poincare´ plot along the line of identity; SDANN, standard deviation of the average of NN intervals in all non-overlapping 5 min segments; SDNN, standard deviation of NN intervals; TI, triangular index; ECG, electrocardiogram; HRV, heart rate variability; PP, P-to-P interbeat intervals; SD, standard deviation; CV, coefficient of variation; BSI, British Standards Institution; RMSSD, square root of mean squared differences between successive NN intervals.

CV, coefficient of variation (in %); BSI, absolute value (in the units of the respective variable) below which the difference between two measurements will lie with 95% probability (following the British Standards Institution).

[a] Missing data from ten 10-min segments due to insufficient number of PP intervals available to calculate SDANN.

Table 3. Reference values for HRV variables, calculated based on 10-h PP and RRf time series (n = 28).

Variable	Unit	PP-based variables				RR_f-based variables				Bland-Altman analysis		
		Mean	SD	Lower Limit of Reference Interval (90% CI)	Upper Limit of Reference Interval (90% CI)	Mean	SD	Lower Limit of Reference Interval (90% CI)	Upper Limit of Reference Interval (90% CI)	Type of Bias	Mean Bias	95% Limits of Agreement
Mean NN	ms	1613	187	1220 (1131-1350)	2008 (1904-2119)	1608	186	1218 (1129-1347)	2003 (1900-2113)	PP - RR_f	5.5	-2.1 to 13
Mean HR	min^{-1}	39	5	31 (29-32)	50 (45-54)	39	5	31 (29-33)	50 (45-55)	PP - RR_f	-0.2	-0.55 to 0.14
SDNN	ms	247	47	161 (147-179)	365 (331-393)	247	45	158 (142-177)	346 (320-370)	PP - RR_f	0.1	-17 to 17
TI		50	12	28 (25-33)	78 (69-86)	60	15	30 (23-38)	93 (84-101)	PP - RR_f	-9.8	-31 to 11
SDANN	ms	169	35	101 (88-116)	245 (222-265)	171	36	107 (96-120)	246 (224-266)	PP - RR_f	-1.8	-8.6 to 5.1
SD2	ms	338	63	209 (182-241)	473 (435-507)	343	61	219 (192-251)	475 (441-507)	PP - RR_f	-4.3	-20 to 12
RMSSD	ms	115	49	45 (40-54)	245 (197-283)	90	31	43 (38-51)	176 (146-208)	PP / RR_f	1.3	0.72 to 1.8
SD1	ms	82	34	32 (28-38)	174 (140-203)	63	22	30 (27-36)	125 (101-148)	PP / RR_f	1.3	0.72 to 1.8

Abbreviations: SD1, standard deviation quantifying the dispersion of data points in a Poincare´ plot perpendicular to the line of identity; SD2, standard deviation quantifying the dispersion of data points in a Poincare´ plot along the line of identity; SDANN, standard deviation of the average of NN intervals in all non-overlapping 5 min segments; SDNN, standard deviation of NN intervals; TI, triangular index; ECG, electrocardiogram; n, number of horses; SD, standard deviation; CI, confidence interval; HRV, heart rate variability; PP, P-to-P interbeat intervals; RMSSD, square root of mean squared differences between successive NN intervals.; RRf, R-to-R interbeat intervals after automated filtering.

Figures

Figure 1. Scatter plots of RR-based, PP-based and RRf-based HRV variables plotted against the total number of 2nd-degree AV blocks over a 10-h ECG recording period. The solid lines represent the linear regression lines, the dotted lines represent the 95% confidence intervals of the regression lines. The *p* values and the coefficients of determination (R2) are listed for each regression analysis. The *p* values listed for ΔPP ('difference to PP'), ΔRRf ('difference to RRf'), and ΔRR ('difference to RR') relate to the pairwise comparison of regression slopes between RR-based, PP-based, and RRf-based HRV variables (level of significance after Bonferroni correction, p=0.017). HRV, heart rate variability; PP, P-to-P interbeat intervals; RR, R-to-R interbeat intervals; RR_f, R-to-R interbeat intervals after automated filtering.

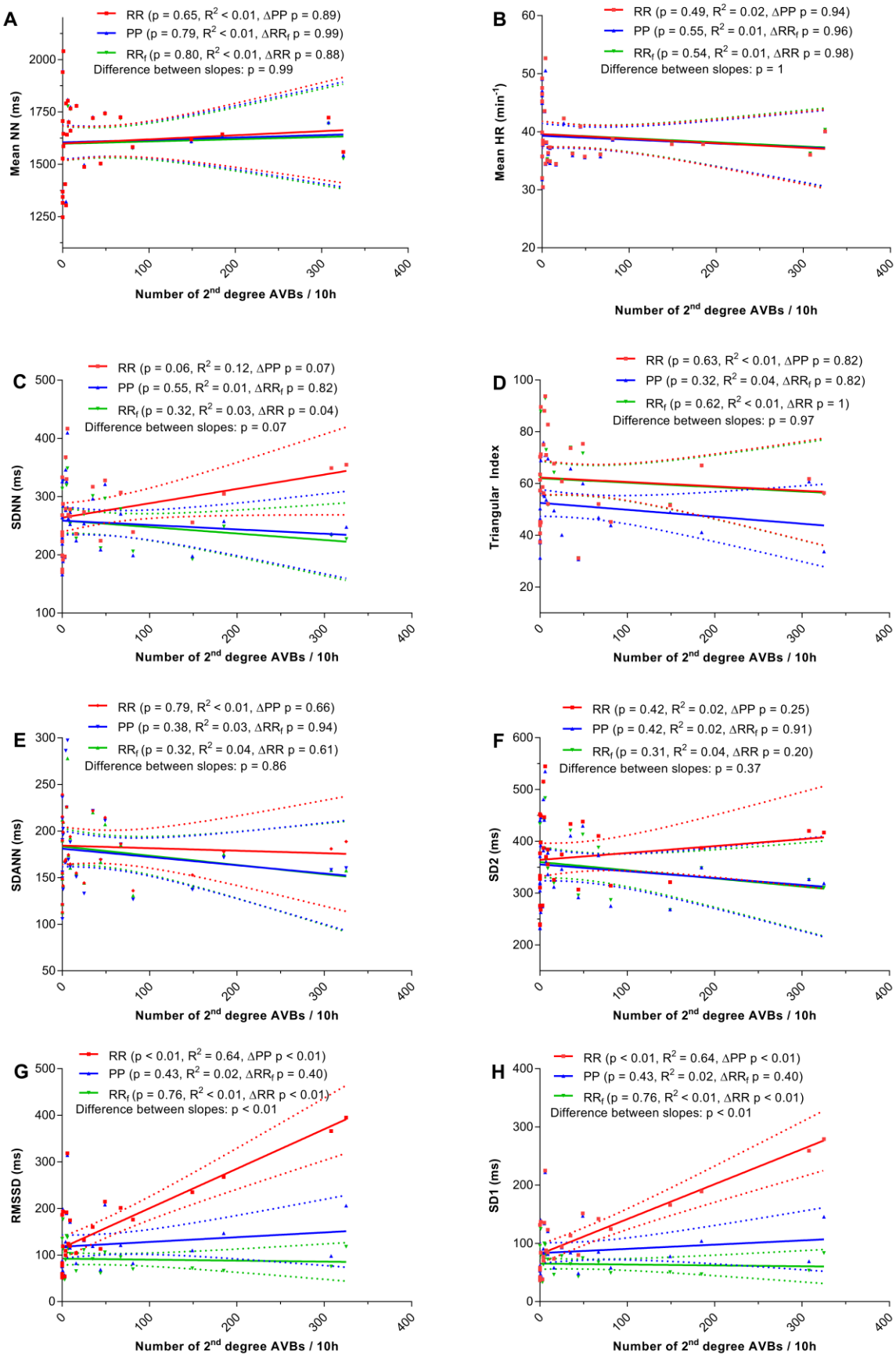

A
RR (p = 0.65, R² < 0.01, ΔPP p = 0.89)
PP (p = 0.79, R² < 0.01, ΔRRf p = 0.99)
RRf (p = 0.80, R² < 0.01, ΔRR p = 0.88)
Difference between slopes: p = 0.99
Mean NN (ms)
Number of 2nd degree AVBs / 10h
B
RR (p = 0.49, R² = 0.02, ΔPP p = 0.94)
PP (p = 0.55, R² = 0.01, ΔRRf p = 0.96)
RRf (p = 0.54, R² = 0.01, ΔRR p = 0.98)
Difference between slopes: p = 1
Mean HR (min-1)
Number of 2nd degree AVBs / 10h
C
RR (p = 0.06, R² = 0.12, ΔPP p = 0.07)
PP (p = 0.55, R² = 0.01, ΔRRf p = 0.82)
RRf (p = 0.32, R² = 0.03, ΔRR p = 0.04)
Difference between slopes: p = 0.07
SDNN (ms)
Number of 2nd degree AVBs / 10h
D
RR (p = 0.63, R² < 0.01, ΔPP p = 0.82)
PP (p = 0.32, R² = 0.04, ΔRRf p = 0.82)
RRf (p = 0.62, R² < 0.01, ΔRR p = 1)
Difference between slopes: p = 0.97
Triangular Index
Number of 2nd degree AVBs / 10h
E
RR (p = 0.79, R² < 0.01, ΔPP p = 0.66)
PP (p = 0.38, R² = 0.03, ΔRRf p = 0.94)
RRf (p = 0.32, R² = 0.04, ΔRR p = 0.61)
Difference between slopes: p = 0.86
SDANN (ms)
Number of 2nd degree AVBs / 10h
F
RR (p = 0.42, R² = 0.02, ΔPP p = 0.25)
PP (p = 0.42, R² = 0.02, ΔRRf p = 0.91)
RRf (p = 0.31, R² = 0.04, ΔRR p = 0.20)
Difference between slopes: p = 0.37
SD2 (ms)
Number of 2nd degree AVBs / 10h
G
RR (p < 0.01, R² = 0.64, ΔPP p < 0.01)
PP (p = 0.43, R² = 0.02, ΔRRf p = 0.40)
RRf (p = 0.76, R² < 0.01, ΔRR p < 0.01)
Difference between slopes: p < 0.01
RMSSD (ms)
Number of 2nd degree AVBs / 10h
H
RR (p < 0.01, R² = 0.64, ΔPP p < 0.01)
PP (p = 0.43, R² = 0.02, ΔRRf p = 0.40)
RRf (p = 0.76, R² < 0.01, ΔRR p < 0.01)
Difference between slopes: p < 0.01
SD1 (ms)
Number of 2nd degree AVBs / 10h

Figure 2. Bland-Altman plots showing the individual data points, the mean bias (solid line), and the 95% limits of agreement (dotted lines). n = 28; two horses that acted as outliers were excluded (see Table 1 and Figures XII, XIII and XIV).

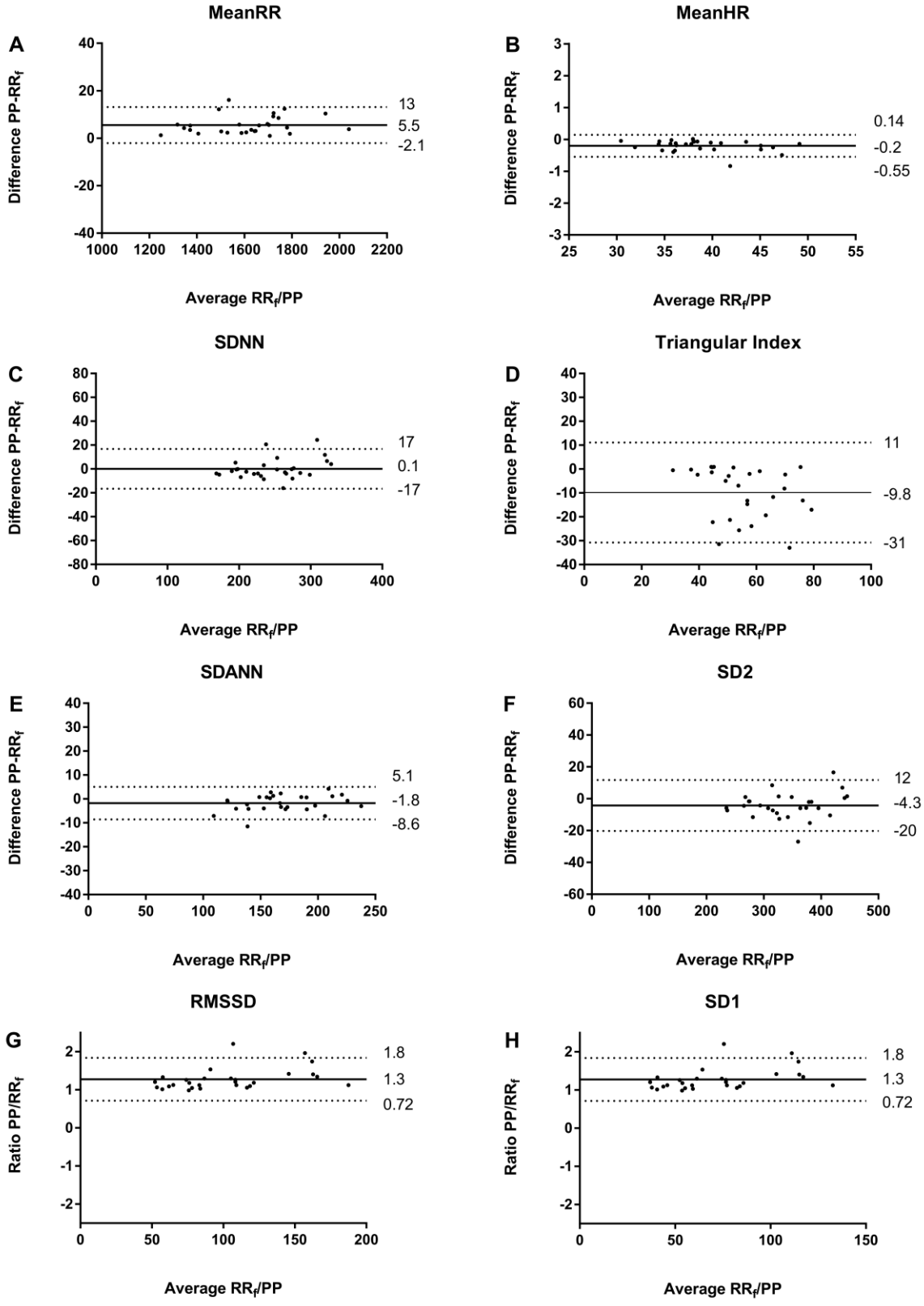
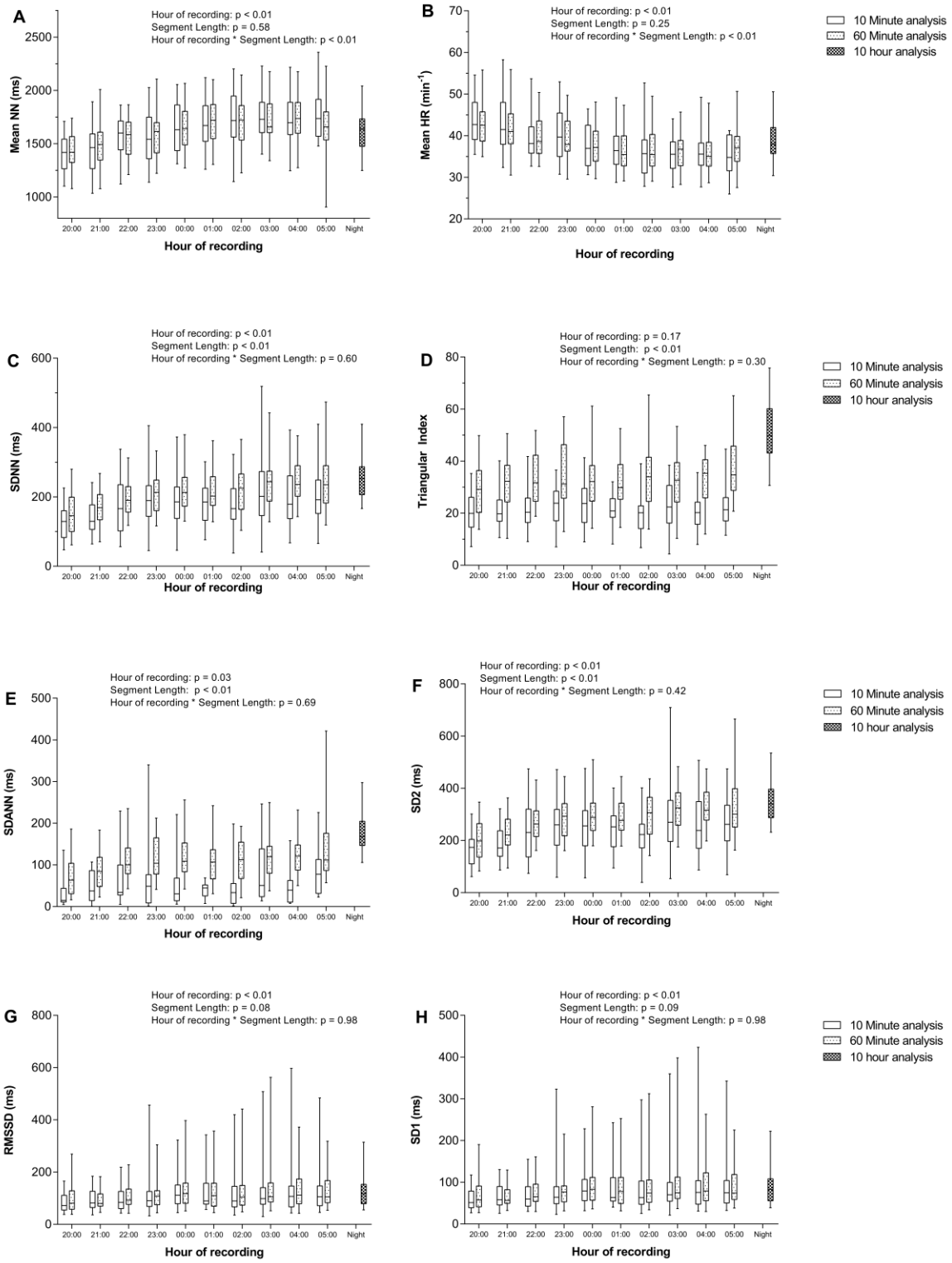


Figure 3. Influence of segment length and recording time on PP-based HRV variables. HRV variables calculated based on 10-min and 60-min ECG segments are plotted over time. The times provided on the x axes represent the starting time of the analyzed time segment; 'night' indicates the respective HRV variable calculated based on the entire 10-h recording, which is provided for comparison. The box plots represent the range between the 25th and the 75th percentile, with the line in the middle of the box plotted at the median. The whiskers indicate the 2.5th and the 97.5th percentile. *p* values are reported for the factors 'hour of recording', 'segment length' and the respective interaction term. The level of significance is $p=0.05$. Pairwise comparisons were not conducted. HRV, heart rate variability; PP, P-to-P interbeat intervals; ECG, electrocardiogram.


A
Hour of recording: p < 0.01
Segment Length: p = 0.58
Hour of recording * Segment Length: p < 0.01
Mean NN (ms)
Hour of recording
B
Hour of recording: p < 0.01
Segment Length: p = 0.25
Hour of recording * Segment Length: p < 0.01
Mean HR (min-1)
Hour of recording
C
Hour of recording: p < 0.01
Segment Length: p < 0.01
Hour of recording * Segment Length: p = 0.60
SDNN (ms)
Hour of recording
D
Hour of recording: p = 0.17
Segment Length: p < 0.01
Hour of recording * Segment Length: p = 0.30
Triangular Index
Hour of recording
E
Hour of recording: p = 0.03
Segment Length: p < 0.01
Hour of recording * Segment Length: p = 0.69
SDANN (ms)
Hour of recording
F
Hour of recording: p < 0.01
Segment Length: p < 0.01
Hour of recording * Segment Length: p = 0.42
SD2 (ms)
Hour of recording
G
Hour of recording: p < 0.01
Segment Length: p = 0.08
Hour of recording * Segment Length: p = 0.98
RMSSD (ms)
Hour of recording
H
Hour of recording: p < 0.01
Segment Length: p = 0.09
Hour of recording * Segment Length: p = 0.98
SD1 (ms)
Hour of recording
10 Minute analysis
60 Minute analysis
10 hour analysis
20:00 21:00 22:00 23:00 00:00 01:00 02:00 03:00 04:00 05:00 Night

Supplemental data

Figure I. Placement of ECG electrodes on the thorax. The LF electrode (green) was placed 5 cm left from the sternum, the LA electrode (yellow) was placed left and approximately 20 cm above the level of the elbow, the N electrode (black) was placed left and approximately 20 cm below the withers and the RA electrode (red) was placed at the same level as the N electrode on the right side of the thorax.

Figure II. Televet screen showing R identification marks and RR intervals after automated RR analysis. Differences in RR intervals indicate sinus arrhythmia. The long pause is caused by a 2nd-degree AVB. The instantaneous heart rate (beats/min) is plotted in red figures at the bottom for all RR intervals.

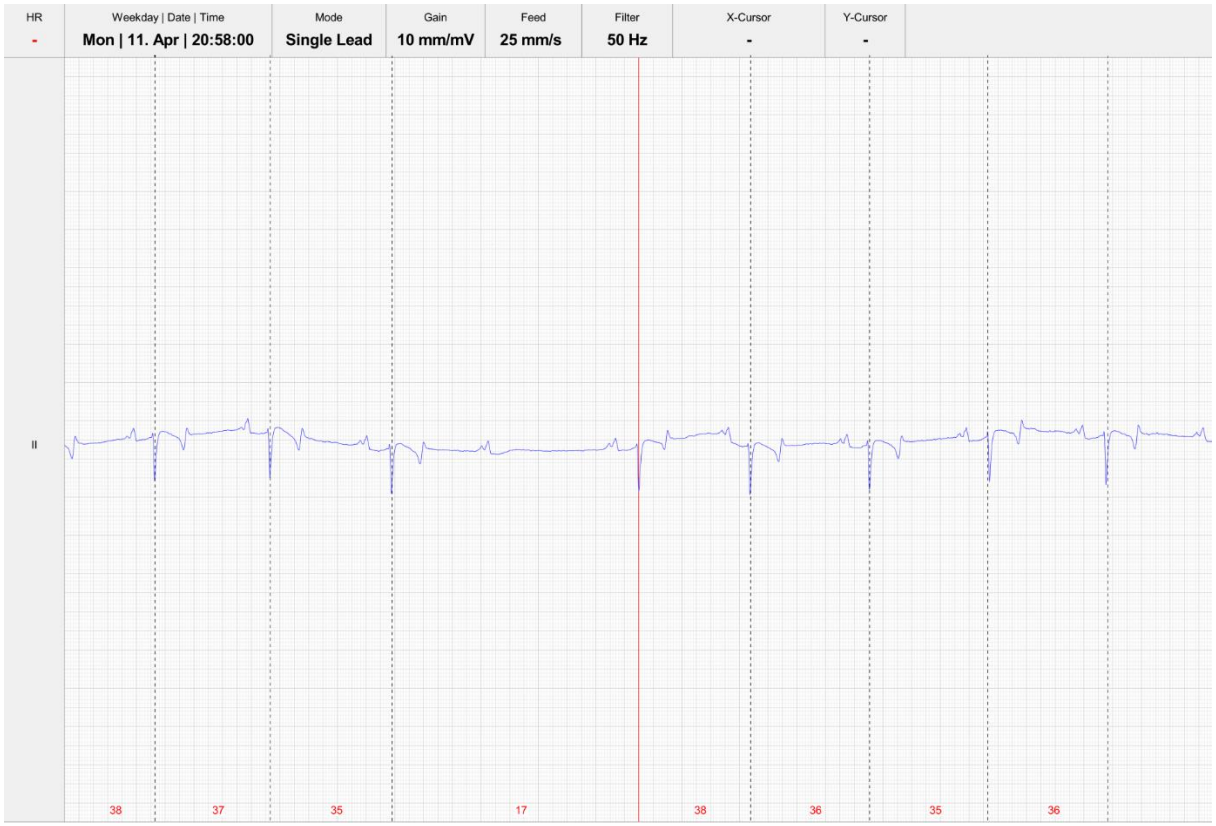


Figure III. Emka TECHNOLOGIES ecgAUTO screen showing the workspace after import of an ECG tracing. The ECG can be checked visually and baseline adjustments and noise removal filters can be set for better analysis quality (not shown).

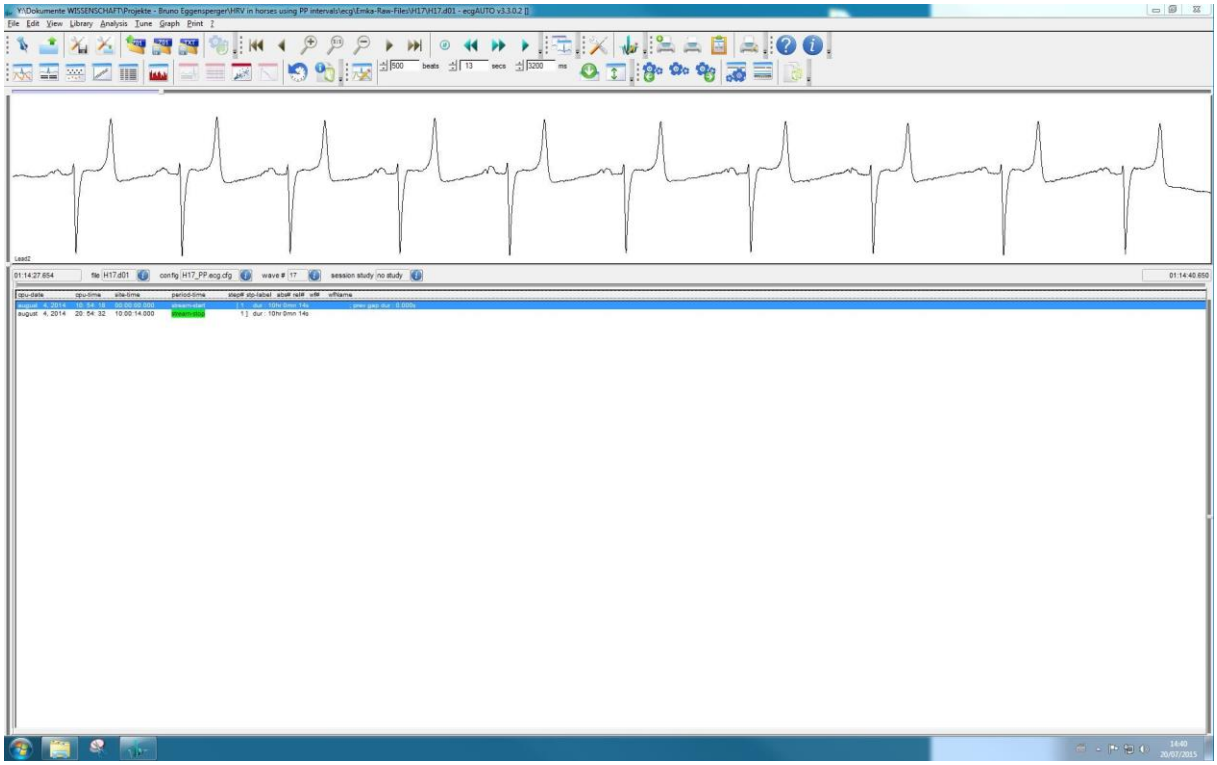


Figure IV. Emka TECHNOLOGIES ecgAUTO screen showing a P-QRS-T complex within the template library builder. Because the software does not support native PP interval analysis, as a workaround the peak of the P wave is marked as ‘R’. This way, P waves can be automatically recognized and are labeled as ‘R peaks’. Hence, the subsequently calculated RR intervals in fact represent PP intervals, which then can be exported for subsequent HRV analysis.

Figure V. Emka TECHNOLOGIES ecgAUTO screen showing an ECG sequence with 10 identified complexes. P waves are marked as ‘R’, PP intervals are labeled as ‘RR’. The individual PP (‘RR’) intervals are listed in the table below. Before analysis of the entire ECG is performed, segments of the ECG can be checked visually to ensure that all complexes are correctly recognized. If necessary, more templates can be defined for complexes with different P-QRS-T shapes and added to a template library (Figure VII).

Figure VI. Emka TECHNOLOGIES ecgAUTO screen after completion of the analysis. The red dots indicate recognized P waves for a one-hour ECG sequence. Below, the software lists all recognized complexes and PP intervals (labeled as 'RR') in a tabular view.

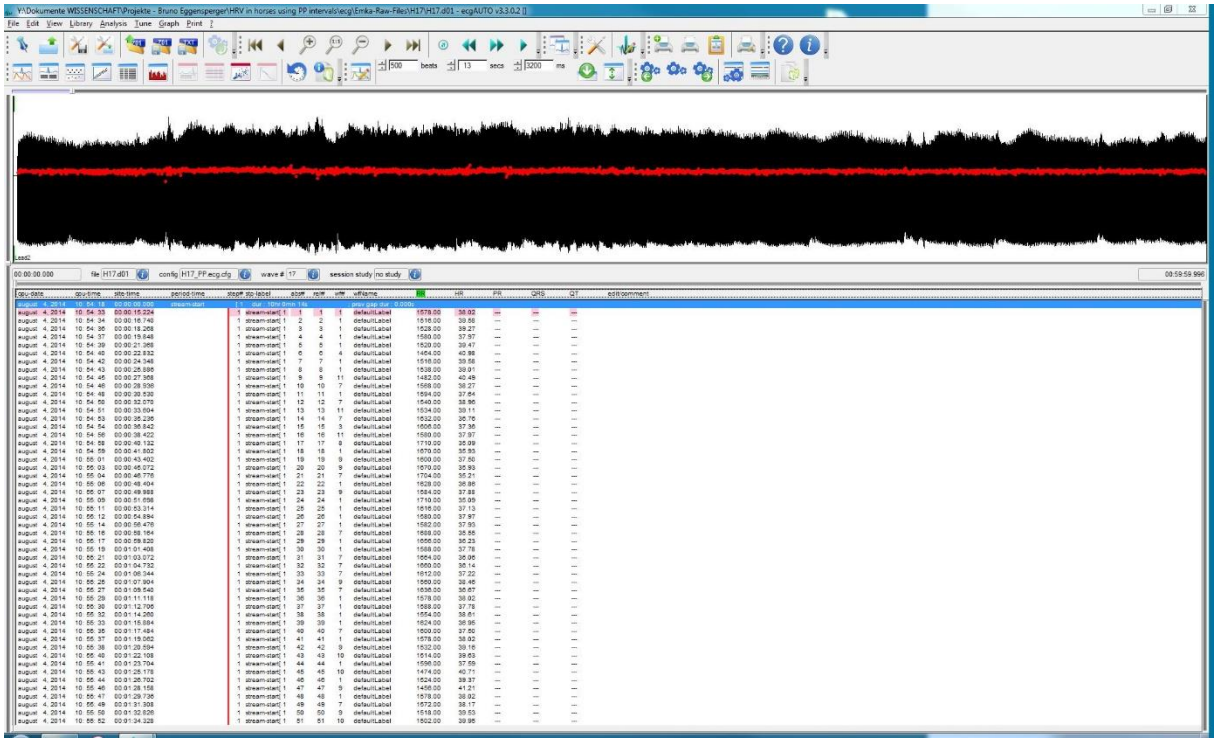


Figure VII. Emka TECHNOLOGIES ecgAUTO screen, showing the template library after analysis of the entire ECG. In this example, 17 templates were required to process 99% of complexes compared to conventional RR interval analysis. The usage count per template is provided in brackets. The ECG complex contained in the template is shown in black, whereas the adjacent complexes are shown in red.

Figure VIII. A: Kubios HRV screen showing a loaded RR interval sequence with the artifact correction filter set to ‘very low’ (red circle). Before pressing the ‘Apply’ button, the effect of the filtering can be seen in the RR tachogram plottet at the top of the screen. The beats that will be removed are colored in blue, the resulting (filtered) RR_f tachogram is displayed as an overlay in green color. The Poincaré plot at the bottom (middle) illustrates the beat-to-beat variability before filtering. B: The same screen after the “very low” artifact filter has been applied. The tachogram (top) and the Poincaré plot (bottom, middle) suggest less variability.

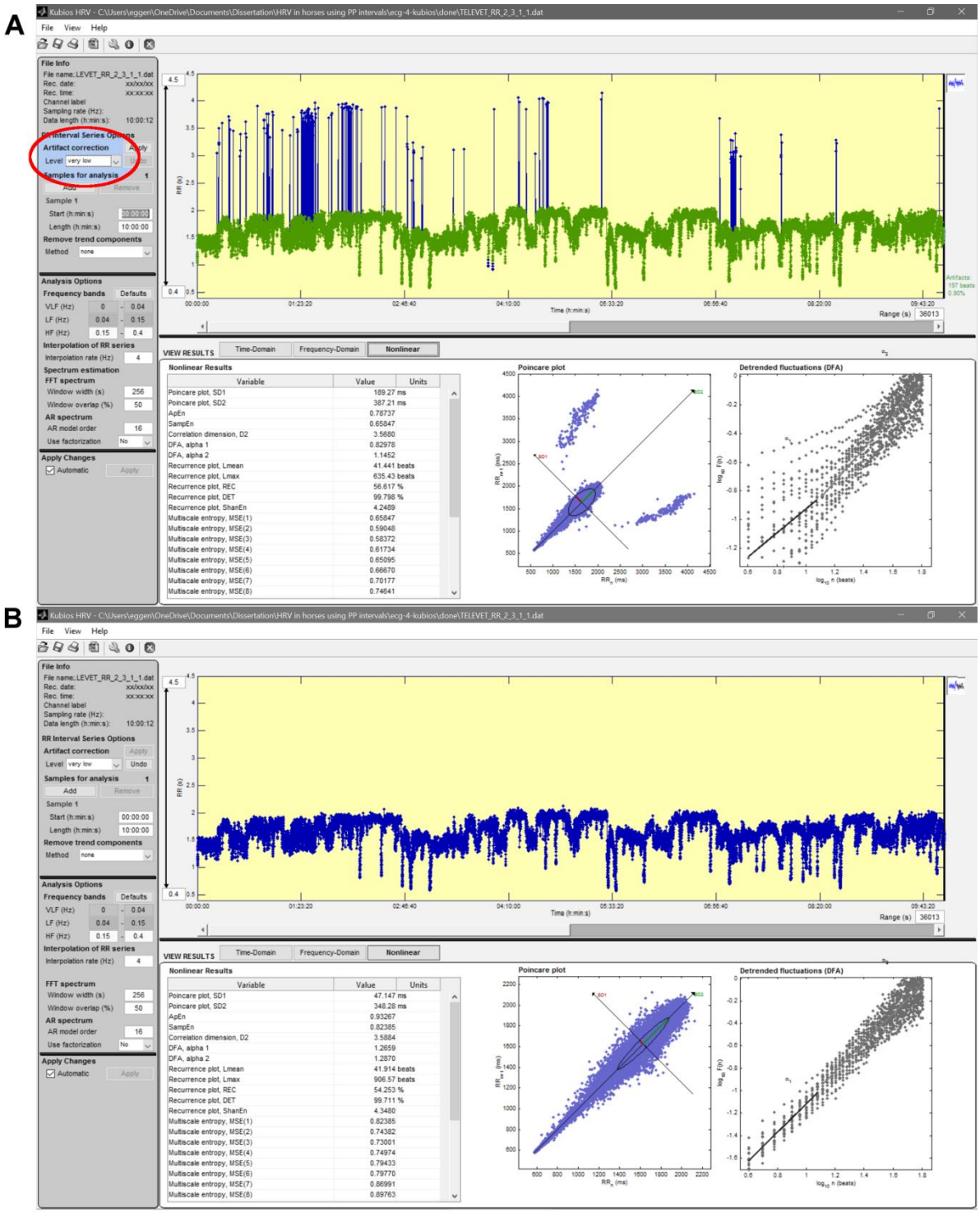


Figure IX. Example of a horse with a total of 325 2nd-degree AV blocks (AVBs) occurring over the entire 10-hour recording period (see Table 1, horse #23). The NN (i.e., RR, PP and RRf) tachograms are displayed on the left, showing all NN intervals (y axis) plotted for all consecutive cardiac cycles (x axis). The histograms of NN intervals are displayed in the middle. The corresponding Poincaré plots, generated by plotting all NN intervals (NN) against the next following NN intervals (NN+1), are displayed on the right.

A: RR interval analysis. The 2nd-degree AVBs can be identified in the RR tachogram as distinct subset of data points at a RR cycle length about twice as long as the regular RR cycle length (gray shaded area). Similarly, the AVBs are displayed in the Poincaré plot as distinct subsets of data points (gray shaded areas). The dispersion of the remaining data points largely represents the heart rate variability caused by sinus arrhythmia as can be seen in Figure X. Due to the relative abundancy of normal sinus beats, AVBs do not become obvious in the corresponding RR histogram.

B: PP interval analysis. The PP tachogram and the corresponding Poincaré plot contain less data points with abnormally long intervals. However, a few remaining prolonged intervals suggest that some P waves were not properly identified (grad shaded areas). In fact, the estimated P wave detection rate in this case was only 97% (see Table 1). The isolated peak seen in the histogram is caused by a technical phenomenon called ‘oscillation’ and is related to an inherent limitation of the software algorithm. Since this peak affects the calculation of the triangular index (leading to a falsely low value), the triangular index was recalculated manually.

C: RRf interval analysis. The RR_f tachogram and the corresponding Poincaré plot show a much narrower dispersion of data compared to the RR interval and the PP interval plots. While all 2nd-degree AVBs were certainly replaced by interpolated beats, some aberrant beats caused by sinus arrhythmia might also have been replaced.

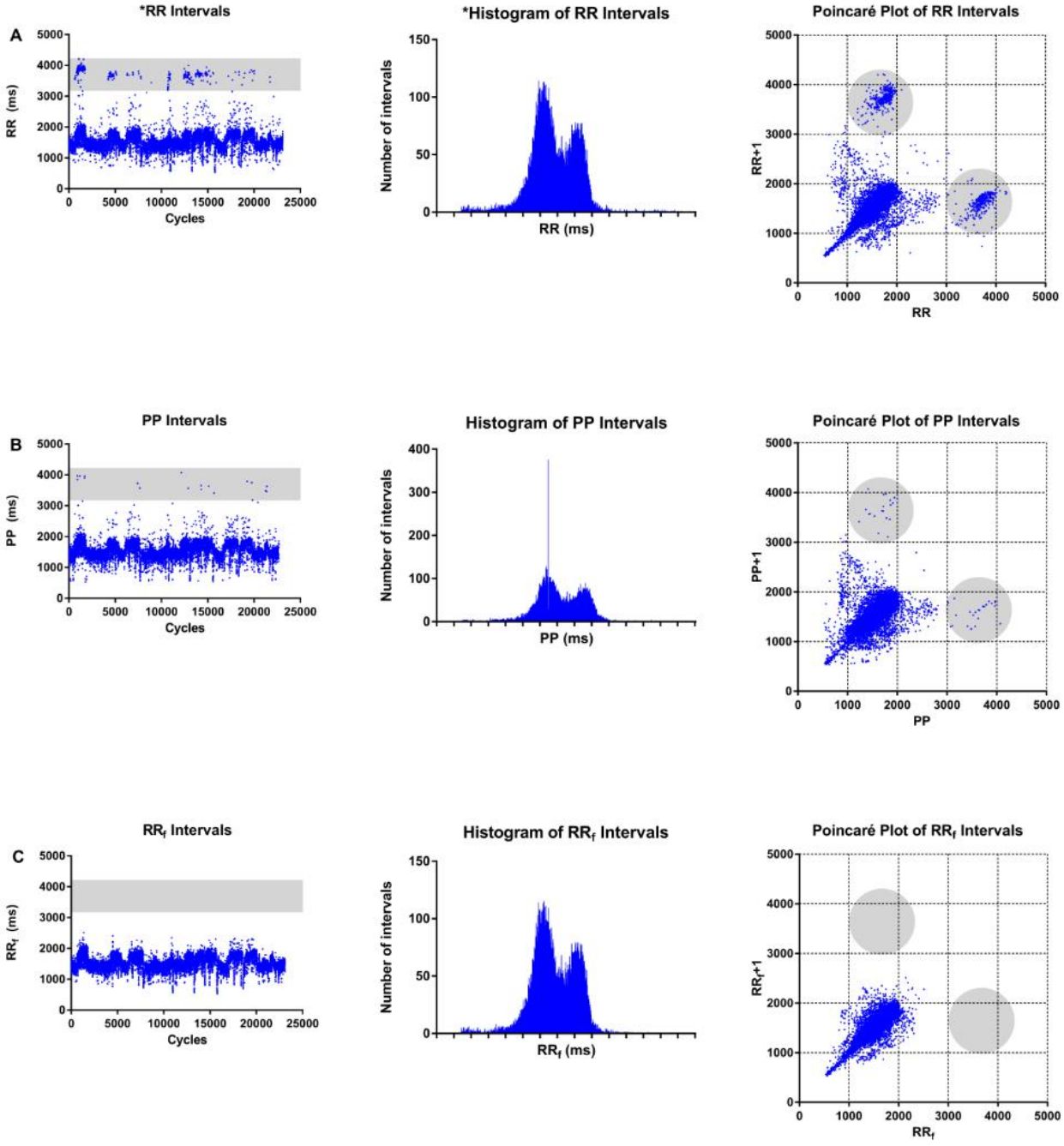

A
*RR Intervals
RR (ms)
Cycles
*Histogram of RR Intervals
Number of intervals
RR (ms)
Poincaré Plot of RR Intervals
RR+1
RR
B
PP Intervals
PP (ms)
Cycles
Histogram of PP Intervals
Number of intervals
PP (ms)
Poincaré Plot of PP Intervals
PP+1
PP
C
RRf Intervals
RRf (ms)
Cycles
Histogram of RRf Intervals
Number of intervals
RRf (ms)
Poincaré Plot of RRf Intervals
RRf+1
RRf

Figure X. ECG sequence showing 2nd-degree AVBs and sinus arrhythmia of the horse analyzed in Figure IX. Red lines indicate that corresponding intervals differed more than 20% from the preceding intervals, resulting from AVBs.

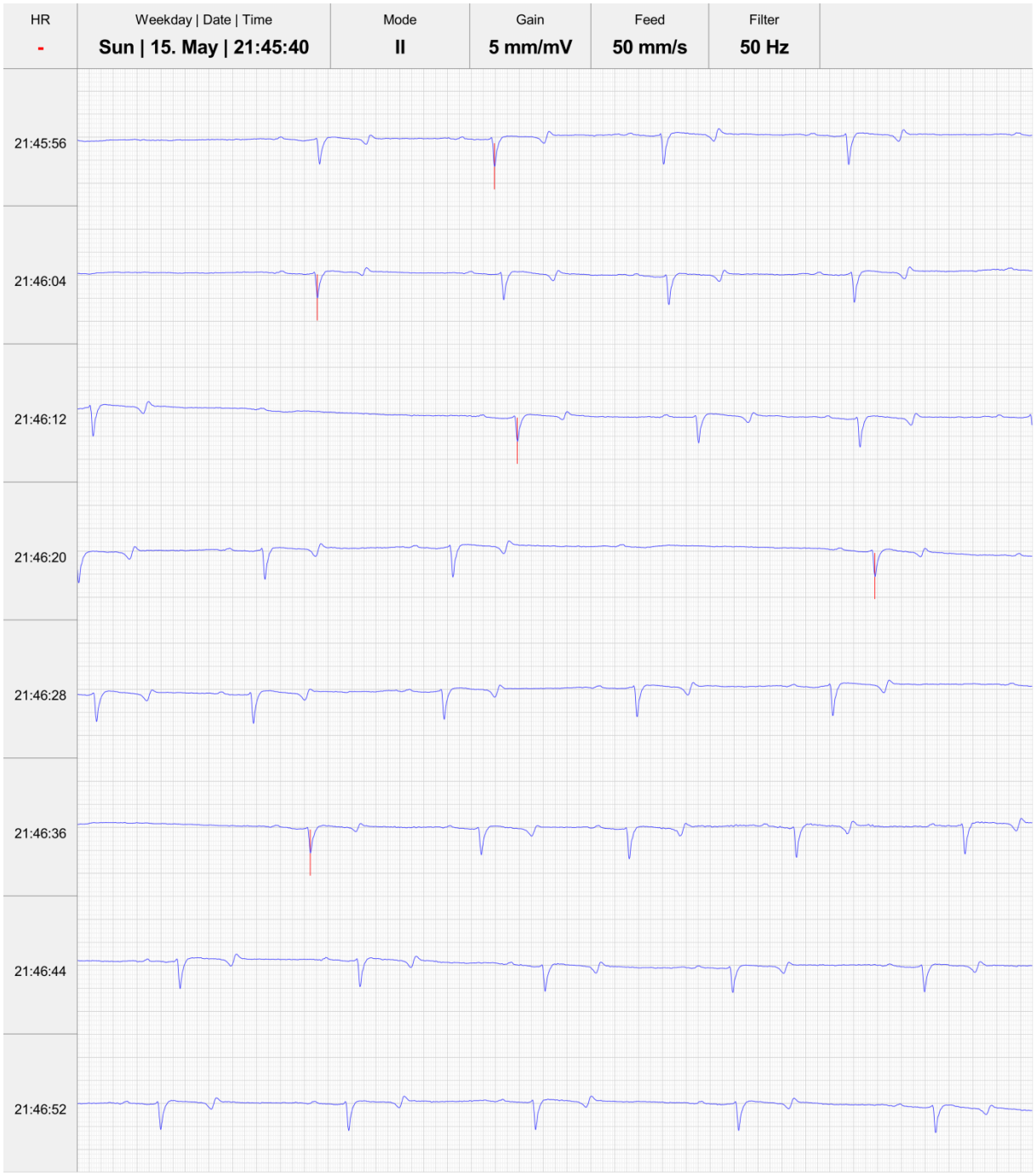


Figure XI. Poincaré Plot showing the dispersion of RR interbeat intervals. SD1 and SD2 are defined as standard deviation of the dispersion of data points perpendicular (SD1) and along (SD2) the line of identity, respectively and determine the width and height of the ellipse shown in the plot.

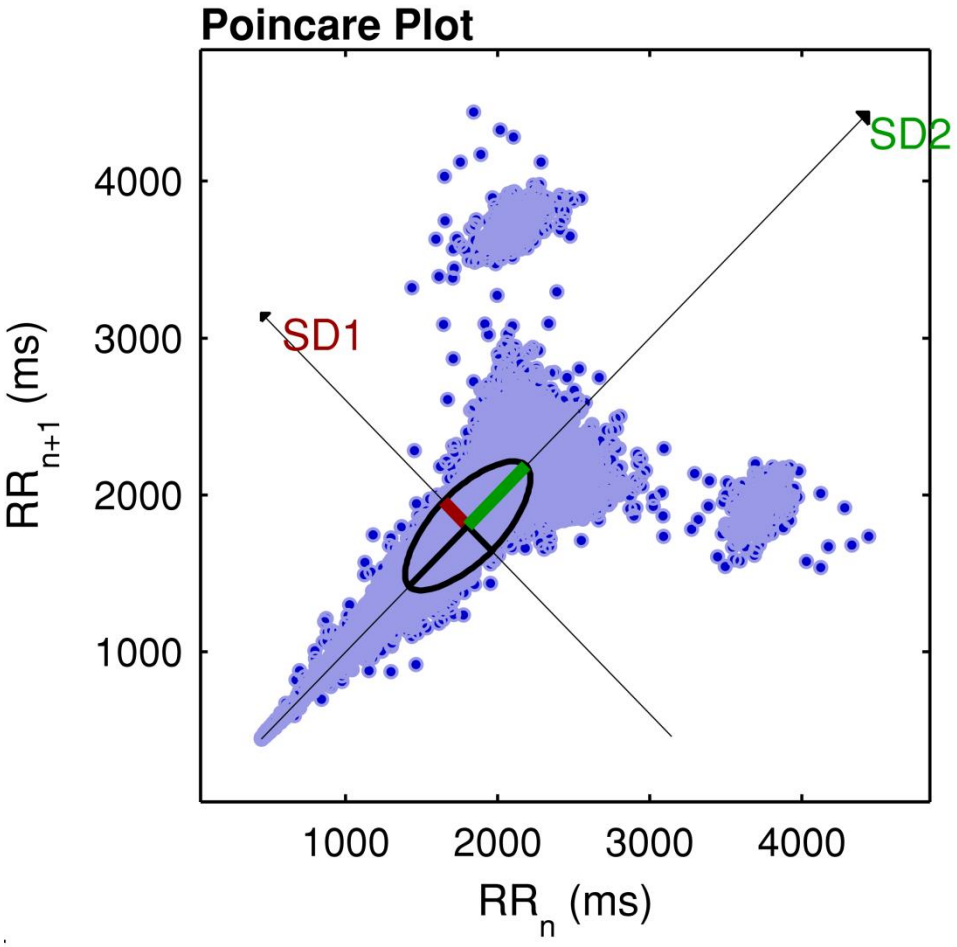


Figure XII. Bland-Altman plots showing the individual data points, the mean bias (solid line) and the 95% limits of agreement (dotted lines). n = 30; two horses act as outliers (#10 and #19, see Table 1 and Figures XIII and XIV). In these two horses, the PP interval time series was markedly influenced by poor P wave detection (horse #10, see Figure XIII) and by large numbers of sinus pauses (horse #19, see Figure XIV).

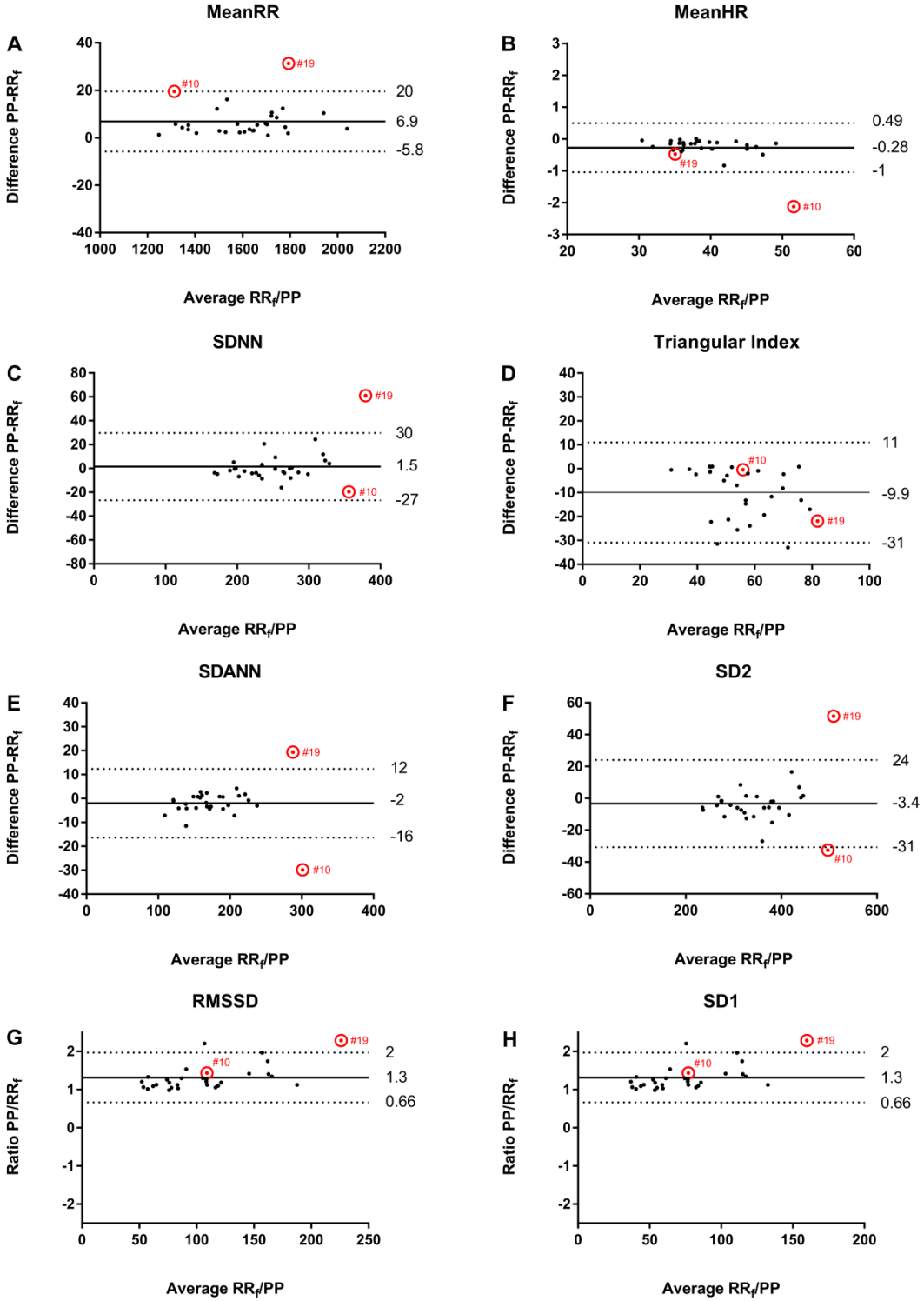
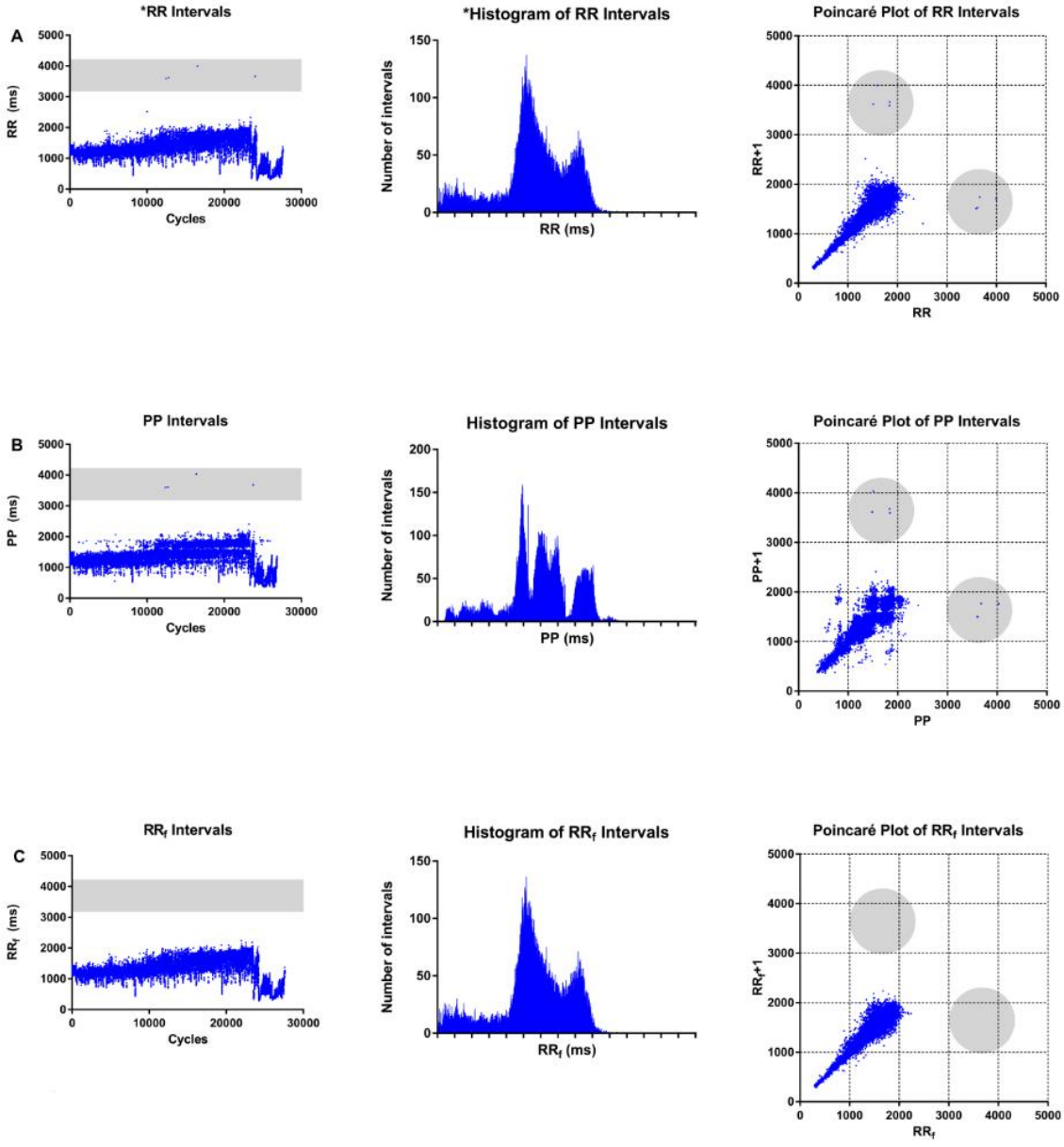


Figure XIII. A: RR tachogram, histogram and corresponding Poincaré plot of horse #10, showing four 2nd-degree AVBs over 10-h (see Table 1). The AV blocks can be identified as a distinct subset of data points (gray shaded areas).

B: Corresponding graphical display of PP analyses, suggesting that the AVBs were not eliminated (gray shaded areas). The estimated P wave detection rate in this horse was 97% (see Table 1). Moreover, an increased degree of sinus arrhythmia was introduced by PP analyses. Inadequate P wave detection and increased NN variability were likely caused by suboptimal P wave recording quality, particularly during phases of sinus tachycardia in the last two hours of recording (see D).

C: Corresponding graphical display of RR_f analyses, indicating that AVBs were adequately removed from the dataset.

D: ECG sequences illustrating sinus arrhythmia (top) and sinus tachycardia (bottom) seen in this horse. Note that the P waves are less distinct and of variable conformation during phases of tachycardia, prohibiting consistent P wave detection by the automated algorithm of the ecgAUTO software.


A
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Cycles
*Histogram of RR Intervals
Number of intervals
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Poincaré Plot of RR Intervals
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RR
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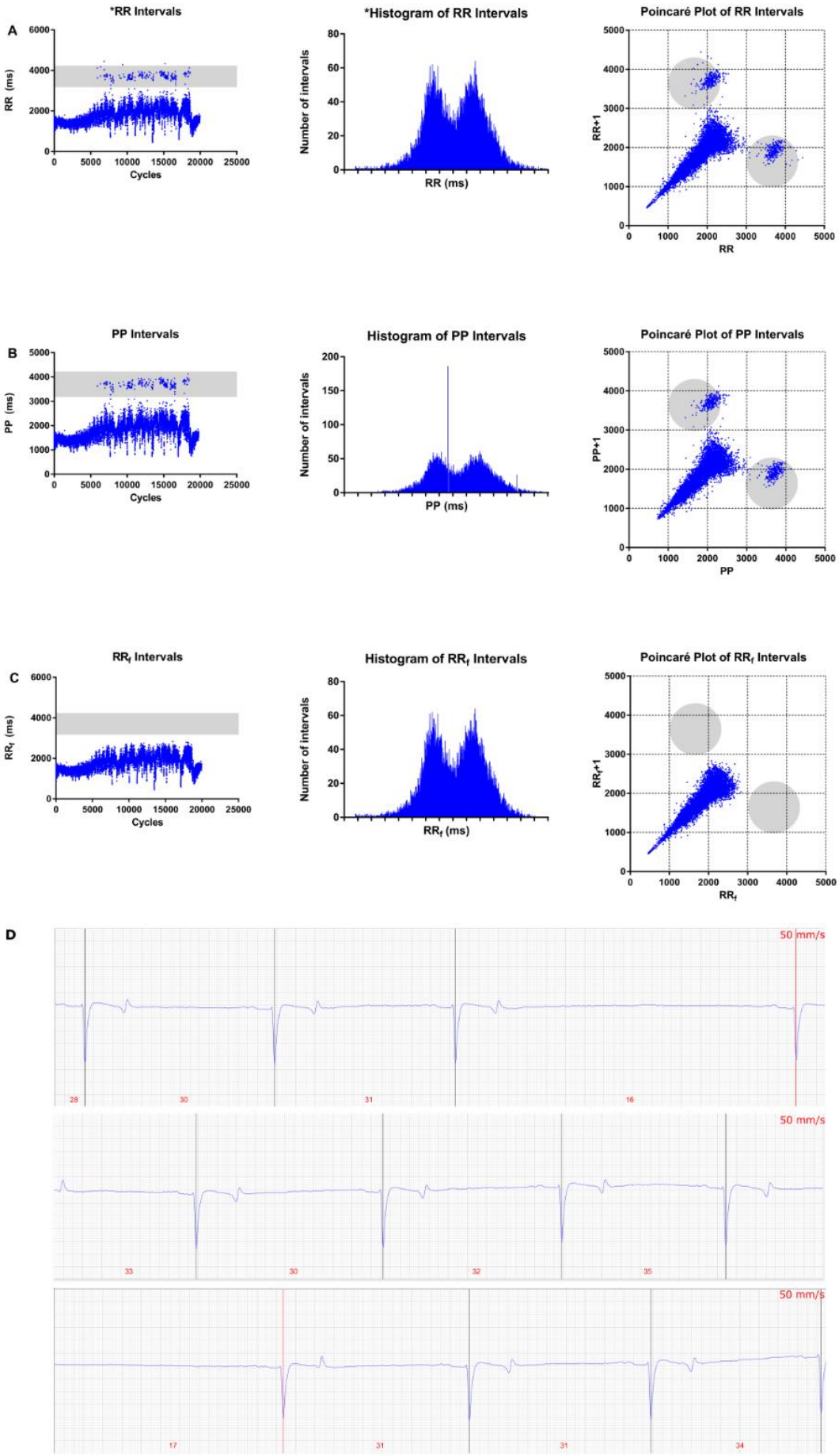
PP Intervals
PP (ms)
Cycles
Histogram of PP Intervals
Number of intervals
PP (ms)
Poincaré Plot of PP Intervals
PP+1
PP
C
RRf Intervals
RRf (ms)
Cycles
Histogram of RRf Intervals
Number of intervals
RRf (ms)
Poincaré Plot of RRf Intervals
RRf+1
RRf
D
50 mm/s
44
31
46
33
39
100 mm/s
141
140
140
142
143
144
145
144
144
143

Figure XIV. A: RR tachogram, histogram and corresponding Poincaré plot of horse #19, which had a total of six 2nd-degree AVBs over 10 hours (see Table 1). This horse also had a large number of sinus pauses (or sino-atrial blocks, see D), mimicking AV blocks in the graphical display (gray shaded areas).

B: Corresponding graphical display of PP analyses. The isolated peak seen in the histogram is caused by a technical phenomenon called 'oscillation' and is related to an inherent limitation of the software algorithm (see text and Figure IX). The sinus pauses still appear as distinct subset of data points (gray shaded areas), indicating that P wave detection was appropriate. The estimated P wave detection rate in this horse was 99% (see Table 1).

C: Corresponding graphical display of RR_f analyses, indicating that not only AVBs but also sinus pauses were eliminated from the dataset.

D: ECG sequences illustrating sinus arrhythmia with sinus pauses seen in this horse.


A
*RR Intervals
RR (ms)
Cycles
*Histogram of RR Intervals
Number of intervals
RR (ms)
Poincaré Plot of RR Intervals
RR+1
RR
B
PP Intervals
PP (ms)
Cycles
Histogram of PP Intervals
Number of intervals
PP (ms)
Poincaré Plot of PP Intervals
PP+1
PP
C
RRf Intervals
RRf (ms)
Cycles
Histogram of RRf Intervals
Number of intervals
RRf (ms)
Poincaré Plot of RRf Intervals
RRf+1
RRf
D
50 mm/s
50 mm/s
50 mm/s

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